Synaptosomal membrane lipids of mice during continuous exposure to ethanol

J. M. LITTLETON*, G. JOHN, *Department of Pharmacology, King's College, University of London, London, WC2R 2LS, U.K.*

The continuous exposure of mice to increasing concentrations of ethanol vapour is associated first with the development of tolerance to ethanol and, after more prolonged administration, with physical dependence on ethanol (Griffiths, Littleton & Ortiz, 1974). The changes in the central nervous system which lead to tolerance and dependence are unknown, but Chin & Goldstein (1977) have reported that tolerance to the physical effects of ethanol can be demonstrated in the isolated synaptic membranes of ethanol-dependent mice. Ingram (1976) has suggested that development of tolerance to ethanol by bacteria follows the incorporation of fatty acids of different degrees of unsaturation into cell membrane phospholipids. Our own results (Abu Murad, Begg & others, 1977; Griffiths, Abu Murad & Littleton, 1977) implicate alterations in lipid metabolism in the development of physical dependence on ethanol. It seemed important to establish whether changes in the fatty acid composition of synaptosomal membranes of mouse brain occurred at any stage during the continuous administration of ethanol.

Mice of the TO Swiss strain (LAB, Dagenham, Essex) were exposed to ethanol vapour in the way previously described (Griffiths & others, 1974). Mice were killed (plunging into liquid nitrogen) either 2 h after commencing inhalation of ethanol, when no signs of physical dependence are seen, or 10 days after commencing inhalation, when withdrawal of ethanol results in a physical withdrawal syndrome. Brains from these ethanol-treated animals were compared with those of mice kept under identical conditions except that ethanol was not present in the inspired air. Ethanol, in the concentration obtained in brains of treated mice, was added to some control brains to establish the effect of the presence of ethanol on the measurement of fatty acids.

Individual whole mouse brains were homogenized in ice-cold 0.32 M sucrose and centrifuged at 800*g* for 10 min. The supernatant was recentrifuged at 25 000*g* for 30 min at 0° and the crude synaptosomal fraction in the pellet subjected to Folch extraction. Phospholipids were adsorbed onto a silicic acid slurry and then eluted with methanol. After alkaline hydrolysis the fatty acids were methylated by boiling for 2 min with boron trifluoride-methanol reagent (BDH). After extraction with chloroform and washing with distilled water, the methylesters were dried under nitrogen and stored in the dark at 4° (maximum period 4 h) before chromatography.

Fatty acid methylesters were separated by gas-liquid chromatography (N_2 as carrier at 70 ml min^{-1} through a 1·6 m glass column packed with 10% polyethylene glycol adipate on Chromosorb WHP). Oven temperature was programmed from 180° to 210° at 2° min^{-1}. Detector (dual flame ionization detectors) temperature was 300°. Retention times and peak areas were obtained from a Vidar 6300 integrator. Peak identification was by comparison of retention times with those of methyl-esterified standard fatty acids (BDH or International Enzymes Ltd) or by comparison with relative retention times in previously published results (e.g. Ackman, 1963).

The main fatty acids found in mouse brain synaptosomal phospholipids are shown in Table 1. The results compare with those given for rat brain by Cotman, Blank & others (1969). In addition to the fatty acids shown, small amounts of other fatty acids were also present. With the exception of a peak thought to represent 24:4 these other fatty acids did not contribute significantly to the total. The fatty acid tentatively identified as 24:4 represented about 2% of the whole, but was difficult to quantify and has therefore been omitted from the analysis. The addition of ethanol to homogenates of control brains produced no alteration in apparent fatty acid composition and these values have therefore been grouped with the controls.

Table 1. *Alteration in fatty acid composition of phospholipids from synaptosomal fraction of mouse brain during continuous administration of ethanol.* Values represent relative peak areas, expressed as a mean percentage of the total ± standard error of the mean, of the fatty acids present (further details see text). n gives the number of observations made in each group. Asterisks indicate a significant ($P < 0·05$ in unpaired Student's *t*-test) difference from controls.

	% fatty acid in synaptosomal phospholipids		
Fatty acid	Control n = 7	Subacute ethanol (2 h) n = 5	Ethanol dependent (10 days) n = 10
16:0	24·24 ± 0·87	26·32 ± 1·12	25·43 ± 1.10
18:0	22.57 ± 0.31	24·12 ± 0·72	24·40 ± 0·61*
18:1	18·21 ± 0·48	18·62 ± 0·54	19·30 ± 0·48
18:2	0·97 ± 0·25	1·16 ± 0·19	1·31 ± 0·23
20:1	2·10 ± 0·12	1·68 ± 0·24	2·20 ± 0·30
20:4	9·94 ± 0·19	8·70 ± 0·33*	8·03 ± 0·31*
22:4	1·87 ± 0·12	1·56 ± 0·23	1·69 ± 0·11
22:6	14·53 ± 0·60	12·86 ± 0·29*	11·86 ± 1·39

Table 1 shows that the continuous administration of ethanol to mice for 2 h or 10 days produces a reduction

* Correspondence.

in the relative proportion off polyunsaturated fatty acids in synaptosomal phospholipids. This shift can be expressed as a single value, the double bond index: saturated ratio. This ratio indexes a proportional change in the fatty acid composition of membrane phospholipids. Its value has been used as a biochemical estimate of the physical fluidity of cell membranes and is closely related to the activity of several membrane-associated enzymes (see Farias, Bloj & others, 1975). The ratio is obtained by dividing the double bond index (the sum of the products of the percentage of each unsaturated fatty acid and the number of its double bonds) by the percentage of saturated fatty acids. For control synaptosomal membranes the double bond index: saturated ratio $\pm$ standard error of the mean is $3{\cdot}393 \pm 0{\cdot}15$, for subacute administration of ethanol the value is $2{\cdot}8000 \pm 0{\cdot}11$, for ethanol-dependent animals it is $2{\cdot}724 \pm 0{\cdot}16$. The double bond index: saturated ratio for ethanol-dependent mice is significantly lower, $P < 0{\cdot}05$ in Student's *t*-test, than that of controls.

These results are consistent with a persistent alteration in synaptosomal composition that is rapidly induced by the continuous administration of ethanol to mice of the TO strain. Whether such a change represents synaptic adaptation to the effects of ethanol on membranes (see Hill & Bangham, 1975) or flows from some toxic or metabolic effect of the drug remains unknown. Chin & Goldstein (1977) have shown that, in ethanol-dependent mice of the DBA strain, tolerance to the physical "fluidizing" effects of ethanol on synaptic membranes occurs. It is possible that changes in membrane lipids of the kind reported here may account for their observations. However, Chin & Goldstein found no change in the intrinsic fluidity of the synaptic membranes from their dependent DBA mice. In preliminary experiments (unpublished) on ethanol-dependent DBA mice we are unable to find any alteration in the fatty acid composition of synaptosomal membranes of this strain. These mice may therefore respond to ethanol in a way different from those of the TO Swiss strain, an observation not inconsistent with known genetic differences in the development of tolerance and dependence (Griffiths & others, 1977).

In conclusion, we report an alteration in the fatty acid composition of synaptosomal phospholipids in the brains of mice undergoing continuous exposure to ethanol. If this change proves to occur at the level of the synaptic membrane, experiments on its time course and genetic basis will be needed to establish its functional significance in ethanol tolerance and dependence.

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REFERENCES

Abu Murad, C., Begg, S. J., Griffiths, P. J. & Littleton, J. M. (1977). *Br.J.exp. Path*, in the press.
Ackman, R. G. (1963). *J.Am.Oil. Chem. Soc.*, *40*, 558–567.
Chin, J. H. & Goldstein, D. B. (1977). *Science*, *196*, 684–685.
Cotman, C., Blank, M. L., Moehl, A. & Snyder, F. (1969). *Biochemistry*, *8*, 4606–4612.
Farias, R. N., Bloj, B., Morero, R. D., Sineriz, F. & Trucco, R. E. (1975). *Biochim. biophys Acta*, *415*, 231–251.
Griffiths, P. J., Littleton, J. M. & Ortiz, A. (1974). *Br. J. Pharmac.*, *50*, 489–498.
Griffiths, P. J., Abu Murad, C. & Littleton, J. M. (1977). *Br. J. Addict.*, in the press.
Hill, M. W. & Bangham, A. A. (1975). *Adv. exp. Med. Biol.*, *59*, 1–9.
Ingram, L. O. (1976). *J. Bact.*, *125*, 670–678.